Neuropeptides 51 (2015) 31–42

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Effect of short and long-term treatment with antipsychotics on orexigenic/anorexigenic neuropeptides expression in the rat hypothalamus

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ARTICLE INFO

Article history:
Received 12 December 2014
Accepted 1 April 2015
Available online 3 April 2015

Keywords:
Neuropeptides
Olanzapine
Chlorpromazine
Haloperidol
Orexigenic
Anorexigenic
Hypothalamus
Rat

ABSTRACT

Among numerous side effects of antipsychotic drugs (neuroleptics), one of the leading problems is a significant weight gain caused by disturbances in energy homeostasis. The hypothalamus is considered an important target for neuroleptics and contains some neuronal circuits responsible for food intake regulation, so we decided to study which hypothalamic signaling pathways connected with energy balance control are modified by antipsychotic drugs of different generations. We created an expression profile of different neuropeptides after single-dose and chronic neuroleptic administration.

Experiments were carried out on adult male Sprague-Dawley rats injected intraperitoneally for 1 day or for 28 days by three neuroleptics: olanzapine, chlorpromazine and haloperidol. Hypothalami were isolated in order to perform PCR reactions and also whole brains were sliced for immunohistochemical analysis. We assessed the expression of orexigenic/anorexigenic neuropeptides and their receptors – neuropeptide Y (NPY), NPY receptor type 1 (Y1R), preproorexin (PPOX), orexin A, orexin receptor type 1 (OX1R) and 2 (OX2R), nucleobindin 2 (NUCB2), nesfatin-1, proopiomelanocortin (POMC), alpha-melanotropin (α-MSH) and melanocortin receptor type 4 (MC4R) – both on the mRNA and protein levels.

We have shown that antipsychotics of different generations administered chronically have the ability to upregulate PPOX, orexin A and Y1R expression with little or no effect on orexigenic receptors (OX1R, OX2R) and NPY. Interestingly, antipsychotics also increased the level of some anorexigenic factors (POMC, α-MSH and MC4R), but at the same time strongly downregulated NUCB2 and nesfatin-1 signaling – a newly discovered neuropeptide known as a food-intake inhibiting factor.

Our results may contribute to a better understanding of mechanisms responsible for antipsychotics' side effects. They also underline the complex nature of interactions between classical monoamine receptors and hypothalamic peptidergic pathways, which has potential clinical applications.

1. Introduction

The pharmacology of antipsychotics (neuroleptics) lends itself to extremely important problems of contemporary neurophysiology and applied psychiatry. Nowadays, neuroleptic receptor-linked mechanisms of action are relatively well documented. There are also some hypotheses concerning the molecular basis of the therapeutical effect evoked by this group of psychotropic drugs, which contribute to a wide range of studies on pathogenesis of depression and schizophrenia, modifying and extending classical dopaminergic and serotoninergic models (Laruelle, 2014; Rogoz, 2013; Schotte et al., 1996). However, commonly used antipsychotics, like olanzapine, clozapine and haloperidol, are characterized by a relatively wide spectrum of side effects. The most important one is an increased risk of patients' weight gain (Leucht et al., 2009a, 2009b), which seems to be particularly high in case of olanzapine and clozapine treatment and depends on serum concentrations of these antipsychotics (Simon et al., 2009).

Body mass regulation is a complex neurophysiological and hormonal phenomenon strictly connected with energy homeostasis control processes performed by specialized hypothalamic areas. Arcuate nuclei (Arc) – groups of neural cells located symmetrically along the ventral part of the third brain ventricle – play a leading and crucial role in energy homeostasis maintenance. This region lacks a blood–brain barrier, so the cells demonstrate sensitivity to the changes in cerebrospinal fluid (CSF) composition (Dietrich and Horvath, 2009; Kohno and Yada, 2012; Norsted et al., 2008), which makes Arc neurons able to respond, by specific receptors, to regulatory hormonal signals like leptin produced in adipocytes, ghrelin produced mainly in endocrinal cells of gastric glands as well as insulin, cholecystokinin (CCK) and peptide YY (PYY). Groups of cells forming Arc are also capable of current

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http://dx.doi.org/10.1016/j.npep.2015.04.001
0143-4179/

glucose concentration sensing and this important feature is probably extended to other substances present in serum, including various pharmaceuticals (Morris and Rui, 2009). In the Arc structure, two functionally opposing, but mutually interdependent neuron populations have been identified. One of them is formed by cells simultaneously synthesizing two factors stimulating food intake, connected with a feeling of hunger (orexigenic factors): neuropeptide Y (NPY) and agouti related protein (AgRP) (Bi et al., 2012; Kohno and Yada, 2012; Nguyen et al., 2014). Moreover, the same neurons express orexin receptors – OX1R and OX2R – and are characterized by a gamma-aminobutyric acid (GABA) coexpression, which proves that they play an inhibitory role in the hypothalamic neuronal network (Abizaid and Horvath, 2008). On the other hand, the second important Arc cell population consists of peptidergic neurons expressing proopiomelanocortin (POMC), its potranslational product-alpha-melanotropin (α-MSH) – and cocaine- and amphetamine-regulated transcript (CART), which belong to satiation factors inhibiting food intake (anorexigenic factors) (Kageyama et al., 2010, 2012; Kohno and Yada, 2012; Lucas et al., 2014). In physiological conditions, these cells are constantly inhibited by GABA, synthetized by neighboring NPY/AgRP neurons (Dietrich and Horvath, 2009; Zigman and Elmquist, 2003), whereas an opposite, stimulating effect on POMC/CART cells is performed by serotonin, which binds to 5-HT_{2C} receptors causing a POMC expression elevation and a subsequent increase in α-MSH synthesis and release (Lam et al., 2008). One of the possible explanations of food intake stimulation in patients treated with olanzapine and clozapine is an antagonistic effect of these pharmaceuticals on hypothalamic 5-HT_{2C} receptors (Kirk et al., 2009; Roerig et al., 2011; Roth et al., 2004). Moreover, in the dorsal part of an Arc, ghrelin expressing neurons have been identified, sending their excitatory projections to NPY/AgRP cells rich in specific growth hormone secretagogue receptors (GSHR) (Kageyama et al., 2010, 2012). The vast majority of axons belonging to Arc neurons reach cells of the paraventricular nucleus (PVN), where they create strong physiological effects with regard to food intake control and thermoregulation. However, some of them head for different hypothalamic regions: ventromedial (VMH), dorsomedial (DMH) and lateral (LH) (Bouret et al., 2004). POMC/CART cells are also able to project their axons directly to the nucleus of the solitary tract (NTS).

Neurons forming PVN and VMH express NPY receptor type 1 (Y1R), melanocortin receptor types 3 (MC3R) and 4 (MC4R), and project their axons to NTS, which consists of cells directly regulating the food consumption process. Furthermore, AgRP synthesized by Arc neurons acts on VMH cells, showing some features of melanocortin receptor antagonist. VMH neurons are also known to express receptors for leptin, insulin, orexins as well as receptors for melanin-concentrating hormone type 1 (MCH_1R). Moreover, PVN and VMH neurons are characterized by histamine receptor types 1 (H1R) and 2 (H2R) expression. Histamine, produced among others in the posterior hypothalamus, is an anorexigenic mediator, so pharmacological blockage of histamine receptors results in significant food intake stimulation and fat accumulation (Chiba et al., 2009; He et al., 2013). Atypical neuroleptics like clozapine and olanzapine act as H1R antagonists, which could be one of the causes of weight gain in psychiatric patients treated with these drugs. There are also suggestions that these substances can be antagonists of histamine receptors type 3 (H3R) identified in the anterior hypothalamus. H3R is primarily a presynaptic autoreceptor of histaminergic neurons, so its activation results in inhibition of histamine synthesis and release. However, it was observed that a number of aminergic and cholinergic neurons express H3R, the stimulation of which causes monoamines and acetylcholine release suppression, resulting in food intake stimulation and body mass increase. Therefore, we can suppose that the orexigenic activity of antipsychotics showing affinity for histamine receptors can be also performed indirectly, as described above (Deng et al., 2010).

On the other hand, LH consists of two interesting and unique types of neurons: cells synthesizing orexins and cells producing a melanin-concentrating hormone (MCH). MCH is a hormone with very strong anorexigenic activity, which is often considered to play a leading role in obesity pathogenesis (Elliott et al., 2004; Ito et al., 2003; Kowalski et al., 2006). It was observed that prolonged MCH injection into rodent CSF causes obesity (Gomori et al., 2003). The latest studies concerning pharmacological strategies for combating obesity focus, to a large extent, on searching for safe and selective MCH1R antagonists and MC4R agonists (Jeon and Cheon, 2009).

Orexigenic neurons are inhibited by NPY and they send retrograde projections to NPY/AgRP neurons of Arc as well as axons heading to NTS (Fu et al., 2004). These cells evoke food intake promoting physiological effects via LH glutamatergic neurons acting through N-methyl-D-aspartate (NMDA) receptors (Doane et al., 2007).

Numerous hypotheses concerning the mechanisms of neuroleptic-related weight gain reflect the complex nature of hunger/satiety regulation. From the pharmacological viewpoint, olanzapine is a dibenzodiazepine derivative that inhibits serotonin 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6, dopamine D_1, D_5, muscarinic M_1, M_5, adrenergic A_1 and histamine H_1 receptors (Roerig et al., 2011; Schotte et al., 1996). At present, the following mechanisms of postneuroleptic body mass increase should be taken into consideration (Bazire, 2005; Esen-Danaci et al., 2008; He et al., 2014; Roerig et al., 2011; Zhang et al., 2013): (1) sedation can decrease daily physical activity promoting weight gain, (2) cholinolytic action of neuroleptics causes thirst increase and subsequent elevated supply of caloric liquids, (3) slowing down of metabolism and decrease in fat and carbohydrate burning, (4) blockage of hypothalamic 5-HT_{2C}, H_1 and perhaps D_1 receptors increases appetite, (5) dysfunctions in neuropeptide and cytokine regulatory factors: leptin, ghrelin, tumor necrosis factor alpha (TNF-α), reductin and cholecystokinin, (6) induction of abdominal fat storage as a consequence of disturbed leptin level, (7) body water retention in the peripheral tissues, (8) hormonal disturbances such as hyperprolactinemia, hypo- and hypercortysolemia and impaired insulin secretion, (9) modulation of the cannabinoid CB_1 receptor activity.

In recent years, we have observed significant progress with regard to explanation and understanding of hypothalamic mechanisms regulating food intake and energy homeostasis. The functional description of cellular interactions within hypothalamic nuclei has been improved, together with a more precise characterization of local neuronal populations and the discovery of new regulatory neuropeptides and their receptors such as orexins, nesfatin-1 and spexin (Palasz et al., 2012; Peyron et al., 1998; Porzionato et al., 2010; Saper and Lowell, 2014). Thus, it is reasonable to expect that particular elements of this complex regulatory system can be modified by neuroleptics. However, at this point, a limited number of studies focus on analysis of the direct impact of neuropsychiatric pharmaceuticals on hypothalamic neuronal centers.

Our study aims to complete this knowledge by determining (on a rat model) if and how selected neuroleptics of different generations and subgroups (olanzapine, chlorpromazine and haloperidol) influence synthesis and release of hypothalamic orexigenic/anorexigenic neuropeptides and expression of their receptors. For the first time, we created a broad expression and distribution profile of different hypothalamic neuropeptides after single-dose and chronic neuroleptic administration. So far, such detailed studies with three different pharmaceuticals administrated in two modes (single vs multiple dose) and with a wide range of neuropeptides have never been conducted on both mRNA and protein levels throughout the hypothalamus. In particular, the newly discovered nesfatin-1 has never before been studied in a pharmacological context. Thus, the expected results should deliver valuable information concerning neuroleptic action at the level of the hypothalamus.